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HIGH-VOLUME CELLULAR TISSUE CULTURE SYSTEMS

TECHNICAL FIELD

Generally, this invention relates to a process for tissue culture generation of plants using support structure systems which may increase the yield of tissue cultured plants, and may even increase the efficiency of labor in performing the tasks related to traditional tissue culture processes and reduce the total process time and may reduce the time of the total process. The present invention may use automated transfer methods and equipment and may increase the effectiveness of plant growth hormones, nutrients and the like provided to the tissue culture plants. Specifically, the invention focuses upon techniques and technology which, in turn, may result in reduced mortality of tissue cultured plants thereby perhaps even increasing a yield of finished tissue cultured plants. The present invention may reduce the number of steps used in traditional tissue culture processes possibly through the use of automated transfer methods and equipment and may provide a more effective method for delivery of plant growth hormones, nutrients and the like to the tissue culture plants. A support structure may allow capillary action for uniform distribution of air, plant hormones and nutrients and may even maximize the development of the tissue cultured plant. The present invention may allow for automated transfer which may reduce labor and may even increase yields of finished tissue cultured products.

BACKGROUND OF THE INVENTION

The use of tissue culture for plant production has been used for many years. Yet, traditional tissue culture may cause high mortality rates and high labor costs. Therefore it may be used currently only for a few high value crops such as exotic tropical plants and flowers, some food crops and even some commercial crops such as lumber. One advantage of tissue culture may be that it may produce an exact phenotypic and genotypic clone of the mother or stock plant that is being tissue cultured. Currently plant breeders and plant production companies may only use tissue culture to produce a very small select group of mother or stock plants which are then propagated using other less expensive methods. They

may only use tissue culture on those species and varieties that may not be propagated using other less expensive methods. Tissue culture may be limited, therefore, to those few crops that can be sold at a premium price to recover the high costs of tissue culture.

The basic tissue culture process may include harvesting a selected small part of a growing mother or stock plant. This small part of the mother or stock plant may be surface sterilized using standard procedures known in the industry. Using sterile equipment in a sterile environmental hood that may have a positive pressure to prevent the inclusion of air borne contaminates, a small part of a mother or stock plant may be cut using a scalpel and forceps. This piece of the small part of the mother or stock plant may be called an explant. Traditionally, each step in the process may require manually handling each explant which may be both labor intensive and may increase the likelihood for the introduction of disease through contamination and explant mortality. The explants may be traditionally placed on a medium containing agar and a predetermined concentration of plant growth hormones and nutrients. The cells of the explants may differentiate on this medium into root and even shoot buds based on the concentration of plant growth hormones and nutrients. This may be called Stage 1.

After a specific amount of time -- which may vary from species to species and variety to variety within a species -- the explants may be transferred to a new medium containing different concentrations of plant growth hormones and nutrients. On this medium the shoot and root buds may be encouraged to develop and grow. This may be called Stage 2.

After a specific amount of time -- which may vary from species to species and variety to variety within a species -- the explants may be transferred again to a new medium containing different concentrations of plant growth hormones and nutrients. On this medium the developed shoot and root may be encouraged to continue to grow until shoot, root and leaves may be clearly visible and the explants mature into plantlets. This may be called Stage 3.

After a specific amount of time -- which may vary from species to species and variety to variety within a species -- the explants may be plantlets and may be transferred to a new container of various sizes that may contain a media that may not include agar in a greenhouse or other non-sterile environment to allow the plantlets to mature and become a new finished plant. This may be called Stage 4. It is well understood by those in the industry that Stage 4 requires some form of support structure to allow for the complete development of roots and shoots to maturity. Stages 1 through 3 may be conducted in the sterile environment of a laboratory using standard tissue culture equipment and techniques. Stage 4 may not be conducted in a laboratory but still may require technical equipment to ensure the successful maturation of the newly formed plantlet from explants. Manual grading of the explants or plantlets may occur between stages to insure that the explants or plantlets that are transferred from one stage to another are uniform in size and development. Uniformity of size and development may greatly increase yield, but manual processing may be expensive and may increase overall production costs.

The sterile medium which may be used in Stages 1 through 3 may not only encourage the transformation of the explants into a plantlet but may also encourage the growth of any contaminates such as fungi and bacteria. Because the size of the explants may usually be very small, any fungi or bacteria or the like that may be present inside or within the explants could grow on the medium indicating that disease or contamination may be present. Therefore, any explants that may survive from Stage 1 to Stage 4 could be considered to be mostly free from fungi and bacterial disease.

Disease in plants is not acceptable. It can diminish the value of a crop by reducing the productivity of the crop through either death of the plant or poor quality finished crops. Many diseases may not be specific to a single species or variety which may allow the spread of disease from the host plant to other plants/crops. Most plants may be propagated using traditional methods which may not be automatically screened for the presence of disease. Since September 11, 2001, the threat to food or other commercial crops through bio-terrorist introduction of disease may have been raised due to awareness of the vulnerability of basic

food and commercial crops to contamination by disease from a host plant that may be imported or native.

The present invention, in embodiments, may focus on a process using a support structure that may allow for the economic tissue culture production of plants. This may allow any plant to be economically produced using this process, not just high value crops. This may decrease the likelihood of the introduction of disease through the traditional propagation method of using a mother plant that may have a disease that has not expressed itself. A diseased mother plant may produce hundreds of diseased plants through traditional propagation methods.

As noted, tissue culture has been used for propagation of plants for many years. There are many different concentrations of different plant growth hormones and nutrients that are used both within a species and/or variety and between species and varieties. The concentration of hormones, nutrients, and the like may vary throughout the tissue culture stages. Several methods have been published using support structures which may reduce labor associated with tissue culture production. These known support structures may not adequately address improving the yield of the finished tissue cultured plants through more uniform distribution of plant growth hormones and nutrients and may not allow for automation during the stages of the tissue culture process.

One type of support structure is noted in International Publication Number WO 87/00394. This publication may describe a support structure made from ceramic fibers. The ceramic fibers may support the explants in Stages 1 through 3 without the need to transfer by hand between each stage. New medium concentrations of plant growth hormones and nutrients may be poured, sprayed or dripped onto the ceramic fibers. Because ceramic fibers may be used as a support structure, the direction of the fibers may be critical to any capillary action of a liquid medium. In addition, the size of the interspatial voids between the fibers may determine the quality of the capillary action of the plant growth hormones and nutrients. Lack of uniformity of both the size of the ceramic fibers and the interspatial voids between the fibers may even result in ununiform or non-uniform distribution of plant growth

hormones and nutrients. The uniformity of distribution of plant growth hormones and nutrients may be important throughout Stages 1 through 3, and may be particularly important in Stage 1 in order to differentiate the cell structure of the explants to form into both shoot and root buds. Ununiform or non-uniform distribution may result in fewer root and shoot bud formations which may decrease the yield or potential of each explants. It may even result in the death of explants possibly due to inadequate plant growth hormones or nutrients. It may also result in uneven growth which cause uneven maturity periods which results in manual grading of explants or plantlets for quality control which is labor intensive and therefore increases labor costs.

Another problem of using ceramic fibers may be that as the fibers may be molded into a size and shape useful for tissue culture production. After the ceramic fibers are molded, they may have to be cut. The compression of the ceramic fibers during the cutting process may fundamentally change the interspatial voids between the fibers and the shape of the terminal or cut end of the fiber. This terminal or cut end may be where the explants rest on the support structure. The cut terminal ends of the fibers may be sharp enough to pierce or perhaps even damage the cell structure of the explants and may reduce the explants vigor. This may increase the length of time for the explants to have cellular differentiation, development of shoot and root buds and maturation from an explant into a plantlet.

The ratio of the surface area of the explants that may be in contact with the support structure may be decreased because the cut end of the fiber may be hard due the nature of ceramic and nonconforming to the shape of the explants. The surface area of the explants that may be in direct contact with the plant growth hormones and nutrients may be reduced with this type of structure. This may result in fewer root and shoot bud differentiation in Stage 1 and may result in poor yields and increased costs. In Stages 2 and 3, root and shoot growth may not be uniformly encouraged possibly resulting again in increased production time and lower yields and ununiform maturity periods which may cause increased production costs.

Because yields in traditional Stage 1 tissue culture may be as low as about 50% or even lower, any additional reduction of yield may greatly increase production costs perhaps even regardless of any labor savings due to fewer transfers between Stages.

During root development in Stages 2 and 3, it may be important that the ratio of air to liquid may be properly maintained so that the roots may not die from drowning. Ununiform or non-uniform interspatial voids due to irregular ceramic fibers and compression of fibers during the cutting of the fibers into a usable shape could cause these ununiform or non-uniform voids to have either too much air or too much liquid thereby possibly reducing the development of roots or even possibly preventing root development into the medium. Lack of root development could increase the time during Stages 2 and 3 and may increase the mortality rate of the plantlet during Stage 4 when the plantlet may no longer be in the controlled environment of the laboratory. This may increase production costs making the process uneconomical.

Another problem may be that a ceramic fiber support structure may not lend itself to automation of transfer from a Stage 3 to Stage 4 environment or perhaps even throughout the tissue culture process. The ceramic fibers may be unidirectional so that it could split or break along directional lines. Automation may require movement and some mechanical equipment to be in contact with a support structure. Because of the small size of a support structure, it might be difficult to have equipment that can move the ceramic fibers without damaging or splitting the ceramic fiber unit. Here, transfers between stages from high density to lower density or even from Stage 3 to Stage 4 may require a manual process. This may increase labor costs and overall production costs.

Another support structure may be described in U.S. Patent No. 4,586,288 to Walton which may use an expanded foam with a gel and a membrane. The membrane may be pierced prior to placing the explants on the surface of the assembly. This piercing process may be done manually. Any manual action may not be consistently reproduced which may result in ununiform or non-uniform piercing of the membrane. The ununiform or non-uniform aperture of the membrane could prevent easy insertion of the explants onto the

medium thereby possibly increasing the time to transfer the explants onto the medium and may increase labor costs. It may also prevent the explants' shoot development from growing upward in a natural way because the shoots may have to pass through the membrane.

The membrane may pose another problem in that it may prevent the uniform distribution of new concentrations of plant growth hormones and nutrients because the membrane may cover the medium. In order for new concentrations of plant growth hormones and/or nutrients to be applied, the old plant growth hormones and nutrients may need to be rinsed from the existing medium. This may require (due to gravity) that the new liquid medium be applied from the top of the support structure and rinsed downward. In this particular assembly, it may not be adequately feasible to rinse the medium in a downward motion due to the membrane. This may prevent the thorough rinsing of a previous concentration of plant growth hormone and nutrient out of the medium.

Because the membrane may be manually pierced, the piercing action could likely also pierce the medium below it. This may result in crushed or damaged medium that could prevent the uniform capillary action of the liquid medium. It could also result in different ratios of surface area of the explants to the surface area of the medium from one explant to another. This could result in uneven differentiation of root and shoot buds during Stage 1 and uneven development of those root and shoot buds during Stages 3 and 4. An increase of time to transfer from Stage 2 to Stage 3 may result because the plantlets may need to be graded by size in order to increase yield in Stage 4. Also, the inconsistency resulting between plantlets could mean that some of the plantlets transferred to Stage 4 could be too immature and could die possibly resulting in decreased yields and increased production costs due to the labor to grade, transfer and then to discard the dead plantlets.

Yet another problem with a membrane may be that because it may cover the entire surface of a medium, it may prevent any automation from occurring. Automation may require easy and complete access to a medium. A membrane could prevent extraction of the support structure by automation thereby increasing labor costs of transfer from Stage 3 to Stage 4 or from transfer from a high density to a lower density area. Further, a membrane

may make manual transfers more difficult because of the need to cut away the membrane without damaging the developing explants and plantlets. This may increase labor costs.

Another problem with the assembly as disclosed in the Walton patent, may be that it may employ a hygroscopic gel which may be both starch based and could attract water. While it may be well understood by those familiar with the tissue culture process that the developing explants and plantlets may require a source of carbohydrates, supplying those carbohydrates in the form of a starchy gel that attracts water may restrict the natural capillary action of the medium. The gel may thereby possibly reduce the effectiveness of plant growth hormones and other nutrients due to ununiform or non-uniform capillary action or ununiform or non-uniform delivery of the required plant growth hormones and nutrients. This could result in slower differentiation of cells into root and shoot buds during Stage 1 and development of those root and shoot buds in subsequent Stages 2 and 3. With the additional source of carbohydrates in the medium, the plant nutrient level may need to be more closely monitored. Before the addition of new concentrations of plant growth hormones and nutrients, the old concentrations of plant growth hormones and nutrients may need to be completely rinsed out in order to be effective. Remaining old plant growth hormones and/or nutrients combinations with new plant growth hormones and nutrients may not produce consistent cell differentiation and subsequent development of root and shoot buds. Without consistent and uniform differentiation and development of root and shoot buds, manual grading of the explants and plantlets may be necessary between each stage possibly increasing labor costs and preventing the opportunity for automation of the transfer process. Increased water availability from the hygroscopic gel may also cause increased water intake by the explant or plantlet which may increase the likelihood of vitrification which is the translucent water soaked succulent appearance which leads to mortality and reduces yields which increases production costs.

Other types of support structures as noted in EP 0692929B1 and similar patents both international and US, may suspend the explants and plantlet on a platform above a liquid medium. The platform base may have a porous material that may allow the liquid medium concentration of plant growth hormones and nutrients to pass through and come in contact

with an explant or plantlet. The problem with this type of support structure may be that the amount of medium and therefore concentration of plant growth hormones and nutrients may be dependent on the porosity of the platform. As the explants and plantlets mature, they may become larger and therefore heavier and may place more downward pressure on the platform. The maturing explants and plantlets may push more of the liquid medium through the pores of the platform.

While some inventions may compensate for an increased pressure on the liquid medium below, there could be potential for inconsistent dispersion of the plant growth hormones and nutrients due to the increased mass of the explants and plantlets and the mechanical action of the floating platform. This may result in an uneven distribution of plant growth hormones and nutrients that could result in ununiform or non-uniform cell differentiation and development of root and shoot buds. This may lower overall yield and may result in the need for manual grading of explants or plantlets that may increase labor costs. Because the developing roots of the explants or plantlets may not be supported, it may be impossible for the process to be automated other than the movement of the entire platform to a new medium. Therefore there may be limited ability to move the developing explants and plantlets from a high density to a lower density. This may result in the need to use a lower density of explants to begin with which may use expensive laboratory or sterile environment space uneconomically. The developing explants could be manually transferred to a new platform at a lower density which may cause increased labor and may increase overall production costs.

DISCLOSURE OF INVENTION

The present invention includes a variety of aspects, which may be selected in different combinations based upon the particular application, or needs to be addressed. In embodiments, the invention may include a support system for tissue culture of plants that may allow for the reduction of labor during the transfer process of Stages 1 through 4. The present invention may employ automated methods and equipment, increased yields of

finished plantlets in all stages and uniform distribution of plant growth hormones, nutrients and the like.

Overall the invention may allow more uniform development of tissue cultured plants which could both increase the yield of finished plants at the end of the process and may allow for automation of procedures throughout the tissue culture process.

In embodiments, the invention provides a uniform support structure that may contain consistent, uniform interspatial or even interstitial void ratios. The support structure may be any material or even media, such as but not limited to organic, inorganic, natural, manmade or the like materials that may be capable of providing a consistent, uniform interspatial void ratio necessary to allow even distribution and delivery of plant growth hormones, nutrients and the like to the explants placed on them. Examples of support structures may include these and other materials which may be properly sterilized and may have the same characteristics of uniform delivery of plant growth hormones, nutrients and the like and could result in uniform differentiation of cells and development of root and shoot buds which may result in increased yields.

In embodiments, interspatial voids may be different sizes but may maintain a size difference of possibly not more than about 25% and a ratio of not less than about 3 to 40 or more than about 5 to 40 large to small voids. Void volume could vary depending on specific species and/or variety requirements based on phenotypic and genotypic requirements of the specific species and/or variety. Void volume may be as low as about 10% or as high as about 60%. This could increase the proper development of root buds during Stage 1 and root formation during Stages 2 and 3. Improved root bud development and root formation could increase yields due to uniform development between explants within a group. This could allow a group of explants to move up to the next stage without grading which may be labor intensive and therefore expensive. This may allow for automation of the transfer between stages. Of course other sizes, size differences, void volumes or even ratios are certainly provided and all should be understood as represented within the scope of this invention.

Another aspect of the invention may be the ratio of height of the support structure to void volume. In order to maintain proper concentrations of plant growth hormones, nutrients and the like at the top and throughout the support structure, it may be necessary to have adequate capillary action of the liquid medium throughout the support structure. Depending on the volume of the voids, the height of the support structure could vary. If a larger void volume is used, a shorter support structure may need to be used because the capillary action with a great void volume could be reduced. In embodiments, the ratio of void volume to height may be between about 7 to about 1 and about 12 to about 1. This ratio may increase uniformity of distribution and delivery of plant growth hormones, nutrients and the like to the explants and plantlets thereby possibly increasing yields of explants and plantlets -- and even reducing production costs. Of course, other ratios are certainly possible and all should be understood as represented within the scope of the invention.

In other embodiments, a support structure may properly support the explants to ensure proper distribution of plant growth hormones, nutrients and the like. Each support structure could have a consistent or uniform pocket or indentation that can cradle the explants much like a pillow cradles a head while sleeping. In embodiments, the contact surface area of the explants to the contact surface area of the support structure could be greater than about 15% and less than about 38%. Again, this could increase the uniformity of development of the explants in each stage and may allow for transfer between stages without grading and could increase yields because immature explants may not be transferred before they have properly developed.

Another goal of the invention may be to reduce labor costs through automation of the transfer between stages. The support structure could provide uniform development of the explants and plantlets which may eliminate the need for manual grading of the explants or plantlets. This could allow for automation of the transfer between stages. Automation could allow for multiple explants or plantlets to be transferred between stages which may greatly reduce labor and production expenses and increase profits. Automation methods and equipment may include processes and procedures that employ machines that may automatically apply new concentrations of plant growth hormones, nutrients and the like both during a specific

stage as well as between stages. Transfer from one stage to another may include processes and procedures that employ machines that may automatically move the support structure and the explants or plantlets located on the support structure to a new location that can allow for new environmental properties of light, humidity and temperature. This equipment may also move the explants from a high density of explants or plantlets per cm^2 to a lower density of explants or plantlets per cm^2 to allow for the natural growth and increased size of the explants as the root and shoot buds develop into plantlets. The equipment may be designed to handle multiple explants or plantlets at a time which may further increase the efficiency of the transfer process. This could greatly improve the efficiency of not only the labor to transfer between stages, but also may reduce the required space in the laboratory or sterile environment that may be by nature of being a laboratory or sterile environment highly expensive and specialized area. Therefore, more explants may be brought to maturity in Stage 4 because of increased uniformity throughout the tissue culture process that may allow for automation and reduced required space during the initial stages of cell differentiation and development of root and shoot buds. This may result in an increase in yield of finished plants at Stage 4 reducing costs overall and increasing potential profits overall. One method of transfer (thought not necessarily the only method of transfer) may be described in International Publication Numbers WO 02/058455 and WO 02/100159 to Tagawa Greenhouses, Inc., hereby incorporated by reference. These publications may describe a process that transfers growing plants or plantlets between stages by punching the plant or plantlet downward through the bottom of a web matrix that may hold the supporting structures with the plants or plantlets. Here, these systems may have proven to be highly successful in the transfer process and could uniquely allow for the transfer of many different stages of explants or plantlets development.

Naturally further objects of the invention are disclosed throughout other areas of the specifications and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1.A-L shows an overview of some of the steps in the tissue culture process using a support structure and automated equipment to transfer between stages.

Figure 1A shows the mother or stock plant.

Figure 1B shows the harvest of a portion of the mother or stock plant.

Figure 1C shows the harvest of a small section of the mother or stock plant making the explants.

Figure 1D shows a cross section close-up of the explants on the support structure.

Figure 1E shows a view of a web matrix of support structures.

Figure 1F shows a close up of the cellular differentiation into root and shoot buds.

Figure 1G shows a web matrix of support structure being automatically rinsed with new medium and the old medium rinsed through the bottom of the web matrix.

Figure 1H shows a close up of root and shoot development in Stage 2.

Figure 1I shows the automated transfer of the initial web matrix of high density to a web matrix of lower density.

Figure 1J shows a close up of root and shoot development in Stage 3.

Figure 1K shows a single support structure transfer from Stage 3 to Stage 4 new media.

Figure 1L shows the automation of a web matrix of support structures of Stage 3 plantlets transferred to Stage 4 finishing media.

Figure 2A-B shows cross sections of the support structure.

Figure 2A shows a cross section of the support structure interspatial voids.

Figure 2B shows a detailed, magnified cross section of voids.

Figure 3A-B shows details of interspatial void volume ratios.

Figure 3A shows a detailed cross section of about 3 to about 40 ratio of large to small voids.

Figure 3B shows a detailed cross section of about 5 to about 40 ratio of large to small voids.

Figure 4A-B shows the ratio of support structure height to interspatial void volume.

Figure 4A shows the ratio of void volume to height of about 7 to about 1.

Figure 4B shows the ratio of void volume to height of about 12 to about 1.

Figure 5A-B shows the pocket of the support structure in relation to an explant.

Figure 5A shows a 3-dimensional view of the support structure without an explant.

Figure 5B shows a cross sectional view of the support structure with an explant.

Figure 6A-B shows detail of the percentage of surface contact between the explant and support structure.

Figure 6A shows a detailed cross section of about 15% surface area contact.

Figure 6B shows a detailed cross section of about 38% surface area contact.

Figure 7A-B diagrams the relationship of the importance of the ratios that influence capillary action to yields.

Figure 7A shows how uniform capillary action may impact uniform distribution of plant growth hormones and nutrients.

Figure 7B shows how uniform distribution of plant growth hormones and nutrients may impact yield.

Figure 8A-B diagrams the impact of support structure on increased yields which allows for automation on profits.

Figure 8A shows how improved support structure results in increased yields which may allow for automation.

Figure 8B shows how automation and increased yields due to improved support structure reduces labor and production costs which may increase profits.

MODE(S) FOR CARRYING OUT THE INVENTION

As mentioned earlier, the present invention includes a variety of aspects, which may be combined in different ways. The following descriptions are provided to list elements and describe some of the embodiments of the present invention. These elements are listed with initial embodiments, however it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described systems, techniques, and applications. Further, this description should be understood to support and encompass descriptions and claims of all the

various embodiments, systems, techniques, methods, devices, and applications with any number of the disclosed elements, with each element alone, and also with any and all various permutations and combinations of all elements in this or any subsequent application. Each of these aspects may at times be discussed separately or at times combined with other aspects in no particular order. It should be understood that all permutations and combinations are possible for any given system.

Figures 1A-L detail various embodiments of the overall tissue culture process using a support structure that could allow both uniform distribution of plant growth hormones, nutrients and the like and allow for the use of automated processes and equipment to reduce labor costs.

An explant (1) may be taken from a mother or stock plant (2) using traditional tissue culture techniques. An explant (1) may be placed on a support structure (3) which may or may not be in a web matrix (4). This process may take place in a laboratory or other sterile environment to prevent contamination of the explant, support structure and medium by air borne contaminates which may cause disease and reduce the potential yield of the explants harvested from the mother or stock plant.

A support structure (3) may be any kind of porous structure. For example, but not being limited to, a support structure may include a non-ceramic fiberous material, a non-gel structure, a foam, such as oasis, and the like structures. In embodiments, a support structure may include, but is not meant to be limited to, peat moss, vermiculite, perlite, expanded foams, fiberous materials, either natural or manmade without unidirectional fibers such as cotton, stabilized organic and inorganic naturally occurring or manmade materials, eligaard or the like materials.

A support structure (3) that an explant (1) may be placed on may be based on the specific species and/or variety requirements for proper development of root (5) and shoot (6) bud differentiation and development. A support structure (3) may physically support a developing explant or plantlet by holding it in a proper orientation to light and perhaps even in an optimal orientation with a liquid medium. In embodiments, a support structure may

even allow physical movement of a support structure with an explant or plantlet. This may facilitate the use of automation (13).

In order for the cells to differentiate into root (5) and shoot (6) buds and then for the root (5) and shoot (6) buds to develop, it may require a correct distribution of plant growth hormones, nutrients and the like to be delivered to an explant (1). In embodiments, distribution of hormones and nutrients may occur through substantially uniform capillary action. Substantially uniform capillary action may require a support structure's (3) internal characteristics to have certain ratios and percentages of size, proportion and relation. Further, in order for root (5) development to occur inside the support structure (3), it may require certain ratios of air to moisture. Again, this may require that a support structure's (3) internal characteristics have certain ratios and percentages of size, proportion and relation.

As shown in Figure 2B, a support structure may have a matrix of a continuous surface (11) filled with interspatial voids (7). The interspatial voids (7) with the continuous surface (11) area may make capillary action possible. In order for the proper distribution of plant growth hormones, nutrients and the like to the explant (1) or plantlet, the correct proportion of continuous surface (11) with interspatial voids (7) may be necessary so that capillary action can occur. The proximity of the continuous surfaces (11) to each other may cause a liquid's capillary action to rise vertically and horizontally through the support structure (3). The size of the interstitial area (12) between the continuous surfaces (11) may depend on the size and volume of the interspatial voids (7). The interspatial voids (7) may not be equal in size or volume and may even vary depending on the type of support structure used. While the size of the interspatial voids (7) may be small (8) or perhaps even large (9), the amount of difference between small (8) and large (9) interspatial voids (7) may not be more than about 25%. This may allow for the proper capillary action necessary to uniformly distribute the plant growth hormones, nutrients and the like to the developing explants (1) or plantlets. In embodiments, the present invention may allow for the rinsing of old medium with new medium as may be necessary to encourage cell differentiation and development of root (5) and shoot (6) buds. The ratio of large (9) interspatial voids to small (8) interspatial voids within the overall volume of interspatial voids (10) may need to be between about 3 to about

40 and about 5 to about 40 in order to maintain proper capillary action and perhaps to evenly distribute plant growth hormones and nutrients as shown in Figures 3A-B.

In embodiments, the present invention may include a shower or even a rinsing system for removal of nutrients, hormones and the like from a support structure. This may include balancing retentive exchange capacities with removal exchange capacities. A rinsing pressure may be greater than a retentive force in order to rinse a support structure of old nutrients, hormones and the like.

With proper distribution and delivery of plant growth hormones through optimum capillary action, the surface area (23) of the explant (1) to the support structure (3) may be critical for allowing the transfer of the plant growth hormones, nutrients and the like to the explant (1) allowing for cell differentiation and development of root (5) and shoot (6) buds.

Some embodiments may include providing a pocket in a support structure which can receive an explant or the like. A pocket may be designed to provide optimal contact of an explant to hormones, nutrients and the like. For example, but certainly not limited to, a pocket may be about 2 or about 3 mm in length and about 1 mm in depth or less. The increased surface area of a support structure that may be in contact with an explant may provide optimal conditions for successful propagation in a tissue culture environment.

The interspatial voids (7) may provide air to the developing roots (5). In embodiments, the present invention may provide balancing air to water in an air volume to water mass ratio. Roots (5) may require a ratio of air to moisture that may be dependent on the individual species and/or variety in order to develop. The ratio of interspatial void volume (10) to continuous surface (11) area could be between about 10% and about 60% depending on individual species and/or variety requirements. With the correct ratios, maximum cell differentiation into root (5) and shoot (6) buds and consequently maximum development of the root (5) and shoot (6) buds may occur. The developing roots (5) may grow into the support structure (3) and may even anchor the explant (1) or plantlet to the support structure (3) and may allow for transfer of the explant (1) or plantlet using the

support structure (3) as a unit that could be consistent for an automated transfer (13) process and equipment.

Optimum capillary action could produce highly uniform explants (1) and plantlets which may facilitate the use of automation (13) for the transfer process. Automatic equipment may require consistent uniformity (14) in order to maintain efficiency. Uniformity could also increase yield (15) of finished (22) plants from the initial explants taken. The higher the yield (15) from beginning to end, the greater the efficiency and the lower the production costs (16) per finished (22) plant may occur. Lower yields may indicate ununiform or non-uniformity which may result in grading by hand based on maturity or characteristics necessary for transfer to the next Stage. This may increase labor costs and may increase overall time which can dramatically increase production costs.

Automation could allow for the easy transfer of multiple explants or plantlets between stages for further increased savings of labor and decreased production costs. In embodiments, uniformity may be critical for automated transfer of multiple explants or plantlets to prevent the transfer of immature or overly mature explants in the same transfer.

Automation could also allow for a more efficient use of expensive laboratory or sterile space during the first stages of the tissue culture process. By utilizing a more dense (17) population spacing, less overall laboratory or sterile area could be required. Then, as the explant or plantlet matures and subsequently becomes larger, the explants or plantlets may be moved to a less dense (18) population spacing which may even allow for uniform development of root and shoot buds.

The invention's attributes of a support structure (3) with uniform capillary action (19) resulting in uniform distribution (20) of plant growth hormones, nutrients and the like may result in increased uniformity (14) of explants and plantlets. This uniformity (14) of explants and plantlets may increase yield (15) which may reduce production costs (16) and may allow for the automation of transfer (13) processes which may reduce labor costs (16) and may even result in an overall increase in profits (21).

In some embodiments, the present invention may include consistent, substantially uniform interspatial voids or even interstitial voids which may result in substantially uniform distribution of dissolved plant growth hormones, nutrients and the like in a liquid medium. Other embodiments may include the maintaining of uniformity of interspatial voids or even interstitial voids and perhaps even including an interspatial void size of about 25%. Interspatial void size ratios may be provided and may be between about 3:40 and about 5:40 large to small voids ratio. Of course any ratio may be included and is meant to be included in the scope of this invention. Interspatial void volume may be provided and may be between about 10% to about 60%. In embodiments, the present invention may provide maintaining proper delivery and distribution of dissolved plant growth hormones, nutrients and the like and may even have a percentage surface contact of between about 15% to about 38% surface of support structure to surface of an explant. An increased uniformity of distribution of plant growth hormones, nutrients and the like may result in increased uniformity of explants and plantlets. An increased uniformity of explants and plantlets may allow for automation of tissue culture processes. In yet other embodiments, the present invention may provide a support structure which may allow developing explants and plantlets to be anchored which may even allow for automated transfer.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves both tissue culture techniques as well as devices to accomplish the appropriate tissue culture. In this application, the tissue culture techniques are disclosed as part of the results shown to be achieved by the various devices described and as steps which are inherent to utilization. They are simply the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this provisional application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly

describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in device-oriented terminology, each element of the device implicitly performs a function. Apparatus claims may not only be included for the device described, but also method or process claims may be included to address the functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims that will be included in any subsequent patent application.

It should also be understood that a variety of changes may be made without departing from the essence of the invention. Such changes are also implicitly included in the description. They still fall within the scope of this invention. A broad disclosure encompassing both the explicit embodiment(s) shown, the great variety of implicit alternative embodiments, and the broad methods or processes and the like are encompassed by this disclosure and may be relied upon when drafting the claims for any subsequent patent application. It should be understood that such language changes and broader or more detailed claiming may be accomplished at a later date (such as by any required deadline) or in the event the applicant subsequently seeks a patent filing based on this filing. With this understanding, the reader should be aware that this disclosure is to be understood to support any subsequently filed patent application that may seek examination of as broad a base of claims as deemed within the applicant's right and may be designed to yield a patent covering numerous aspects of the invention both independently and as an overall system.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. Additionally, when used, the term “element” is to be understood as encompassing individual as well as plural structures that may or may not be physically connected. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it

should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "support structure" should be understood to encompass disclosure of the act of "supporting" -- whether explicitly discussed or not -- and, conversely, were there effectively disclosure of the act of "supporting", such a disclosure should be understood to encompass disclosure of a "support structure" and even a "means for supporting." Such changes and alternative terms are to be understood to be explicitly included in the description.

Any patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. Finally, all references listed in the list of References To Be Incorporated By Reference In Accordance With The Provisional Patent Application or other information statement filed with the application are hereby appended and hereby incorporated by reference, however, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

Thus, the applicant(s) should be understood to have support to claim and make a statement of invention to at least: i) each of the tissue culture systems as herein disclosed and

described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) each system, method, and element shown or described as now applied to any specific field or devices mentioned, x) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, xi) the various combinations and permutations of each of the elements disclosed, and xii) each potentially dependent claim or concept as a dependency on each and every one of the independent claims or concepts presented.

With regard to claims whether now or later presented for examination, it should be understood that for practical reasons and so as to avoid great expansion of the examination burden, the applicant may at any time present only initial claims or perhaps only initial claims with only initial dependencies. Support should be understood to exist to the degree required under new matter laws -- including but not limited to European Patent Convention Article 123(2) and United States Patent Law 35 USC 132 or other such laws-- to permit the addition of any of the various dependencies or other elements presented under one independent claim or concept as dependencies or elements under any other independent claim or concept. In drafting any claims at any time whether in this application or in any subsequent application, it should also be understood that the applicant has intended to capture as full and broad a scope of coverage as legally available. To the extent that insubstantial substitutes are made, to the extent that the applicant did not in fact draft any claim so as to literally encompass any particular embodiment, and to the extent otherwise applicable, the applicant should not be understood to have in any way intended to or actually relinquished such coverage as the applicant simply may not have been able to anticipate all eventualities; one skilled in the art, should not be reasonably expected to have drafted a claim that would have literally encompassed such alternative embodiments.

Further, if or when used, the use of the transitional phrase "comprising" is used to maintain the "open-end" claims herein, according to traditional claim interpretation. Thus, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible.

Finally, any claims set forth at any time are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right to use all of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of the incorporated content of such claims or any element or component thereof from the description into the claims or vice-versa as necessary to define the matter for which protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or treaty, and such content incorporated by reference shall survive during the entire pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.

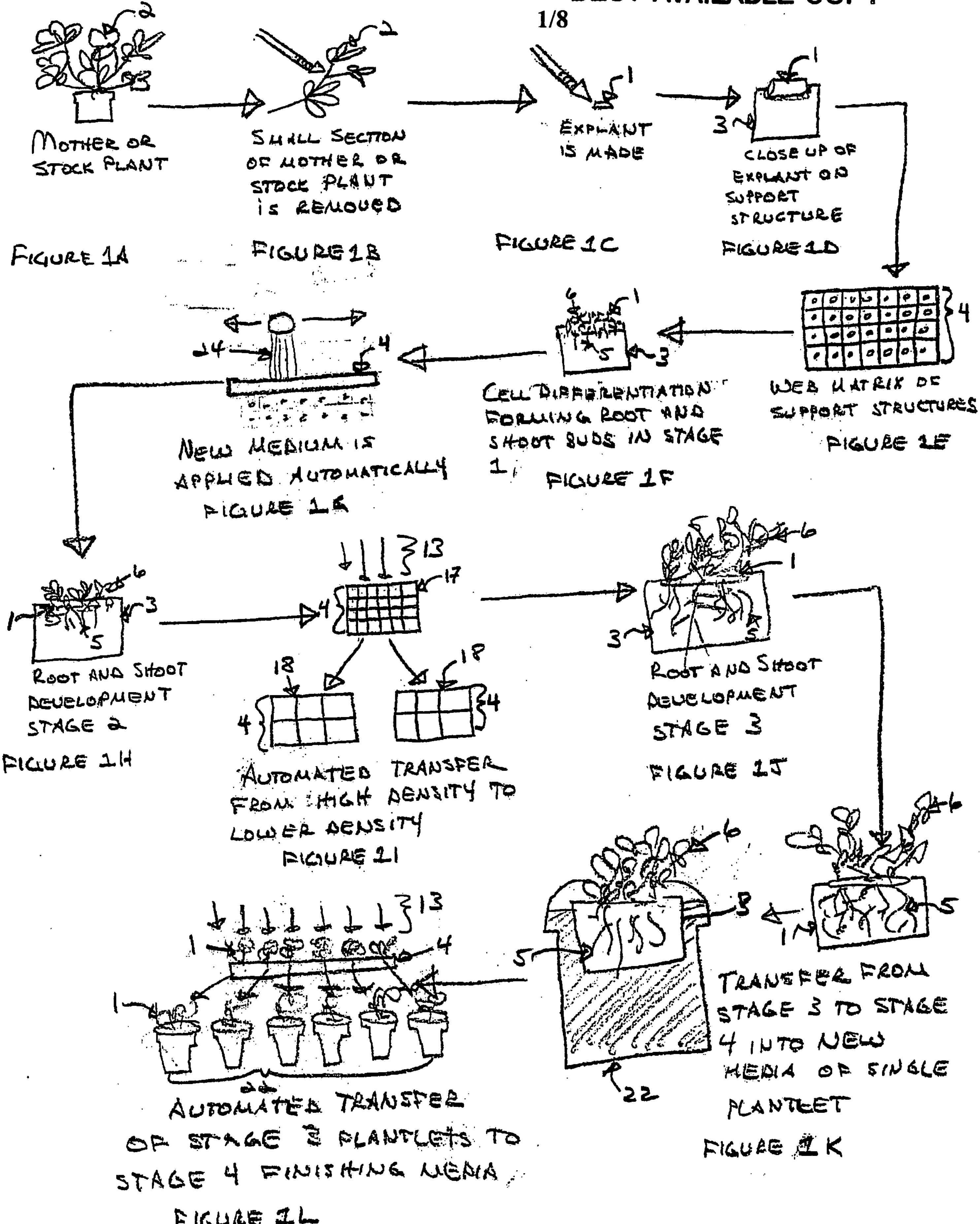
MOTHER OR STOCK PLANT
FIGURE 1A
SMALL SECTION OF MOTHER OR STOCK PLANT IS REMOVED
FIGURE 1B
EXPLANT IS MADE
FIGURE 1C
CLOSE UP OF EXPLANT ON SUPPORT STRUCTURE
FIGURE 1D
WEB MATRIX OF SUPPORT STRUCTURES
FIGURE 1E
CELL DIFFERENTIATION FORMING ROOT AND SHOOT BUDS IN STAGE 1
FIGURE 1F
NEW MEDIUM IS APPLIED AUTOMATICALLY
FIGURE 1G
ROOT AND SHOOT DEVELOPMENT STAGE 2
FIGURE 1H
AUTOMATED TRANSFER FROM HIGH DENSITY TO LOWER DENSITY
FIGURE 1I
ROOT AND SHOOT DEVELOPMENT STAGE 3
FIGURE 1J
TRANSFER FROM STAGE 3 TO STAGE 4 INTO NEW MEDIA OF SINGLE PLANTLET
FIGURE 1K
AUTOMATED TRANSFER OF STAGE 3 PLANTLETS TO STAGE 4 FINISHING MEDIA
FIGURE 1L

FIGURE 1A-L

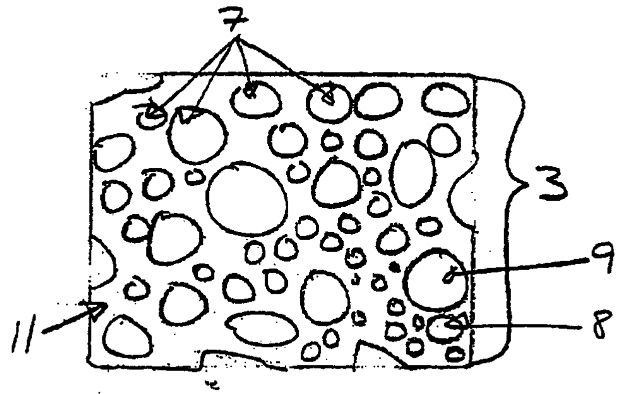

FIGURE 2B

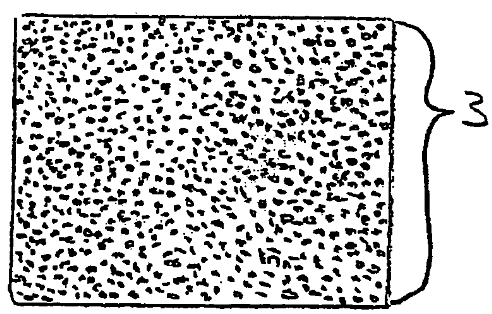

FIGURE 2A

FIGURE 2A-B

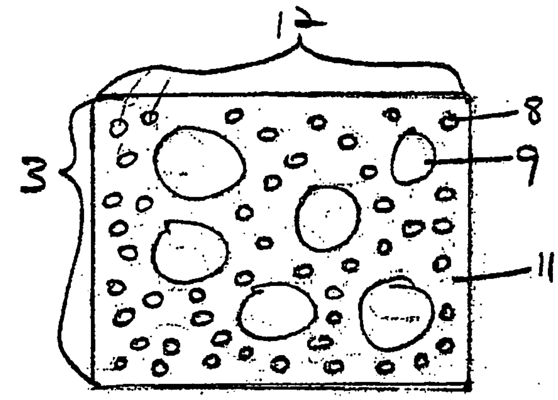

FIGURE 3B

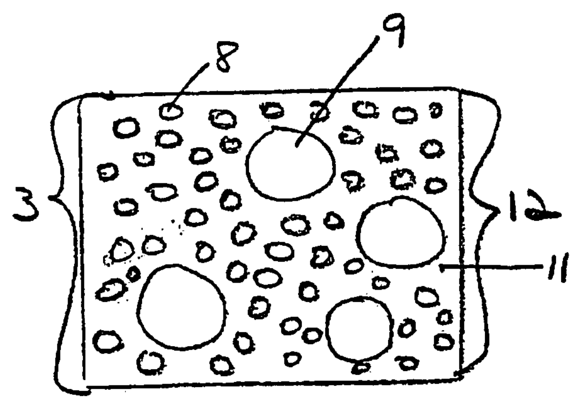

FIGURE 3A

FIGURE 3A-B

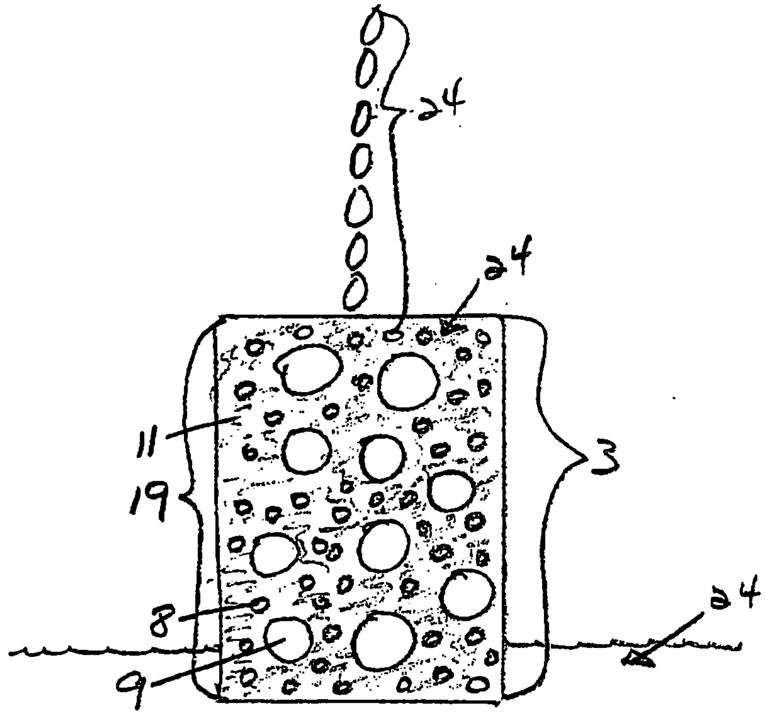

FIGURE 4B

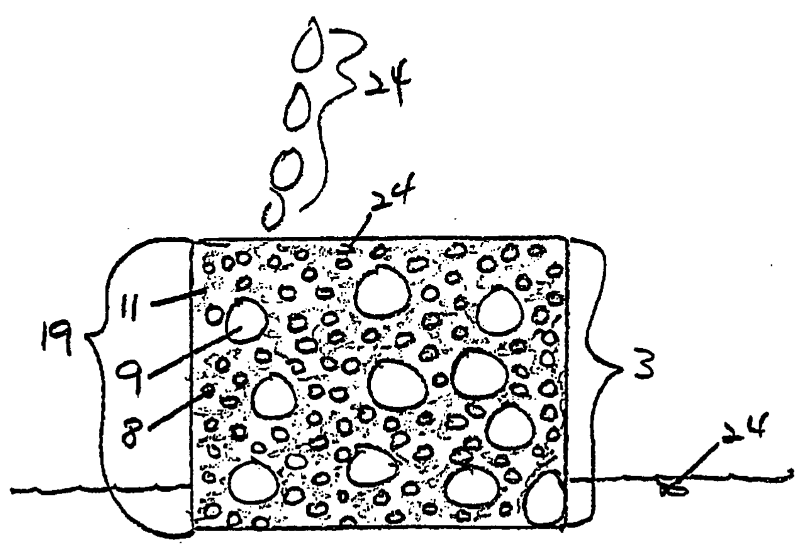

FIGURE 4A

FIGURE 4A-B

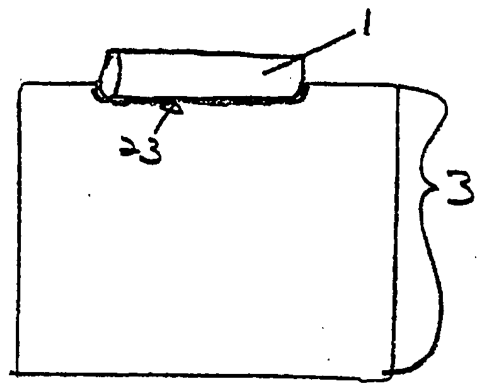

FIGURE 5B

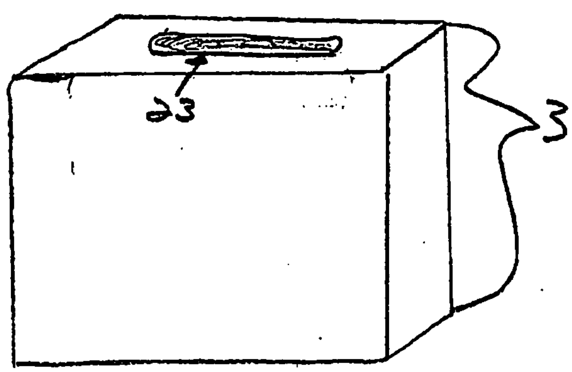

FIGURE 5A

FIGURE 5A-B

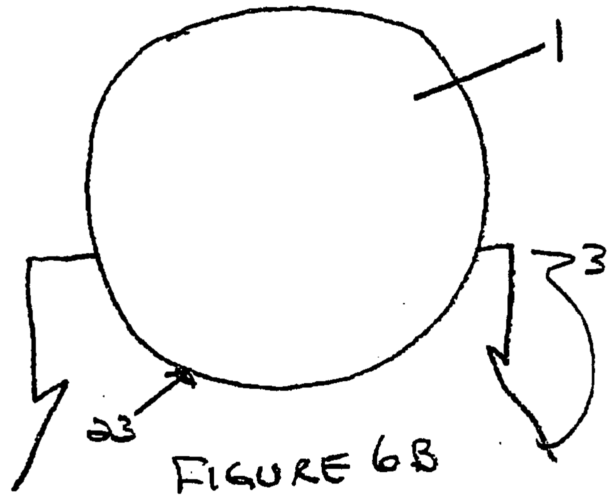

FIGURE 6B

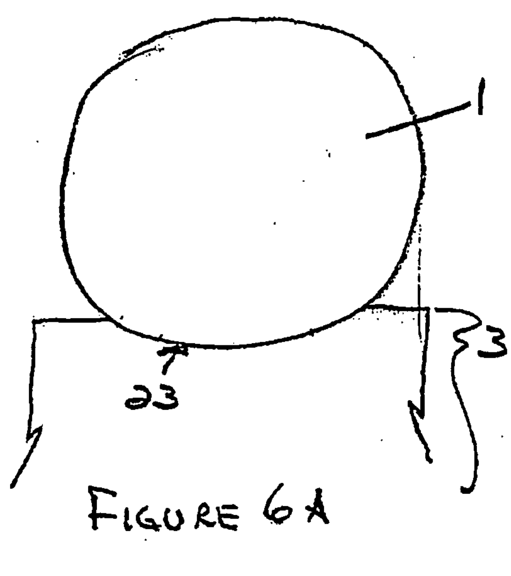

FIGURE 6A

FIGURE 6A-B

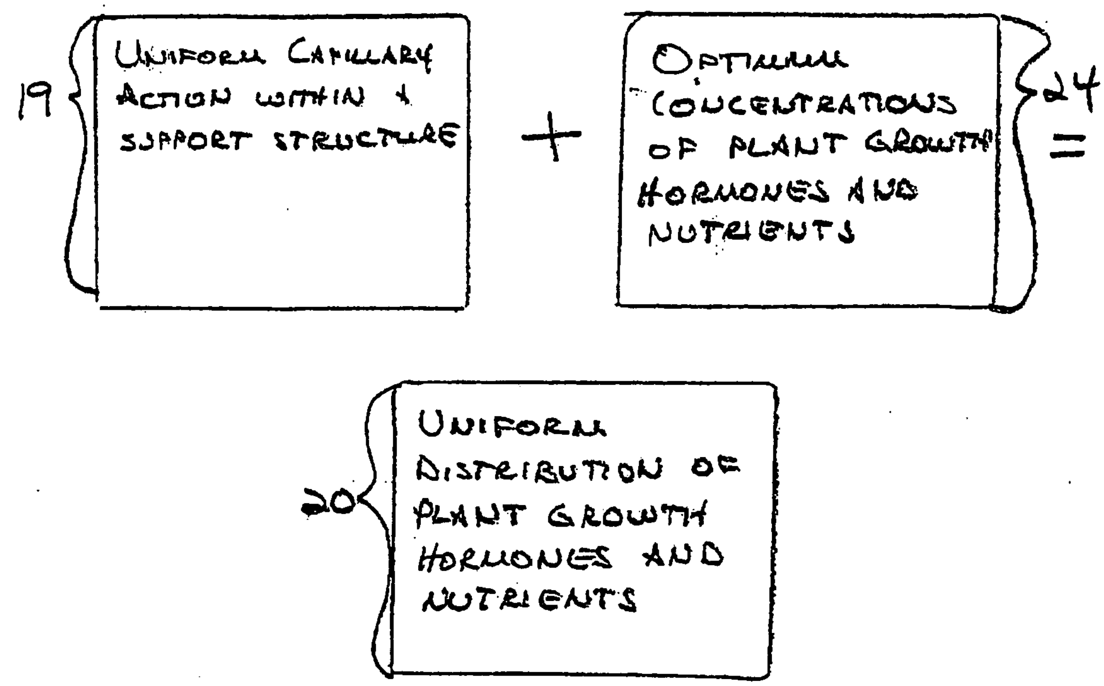

FIGURE 7A

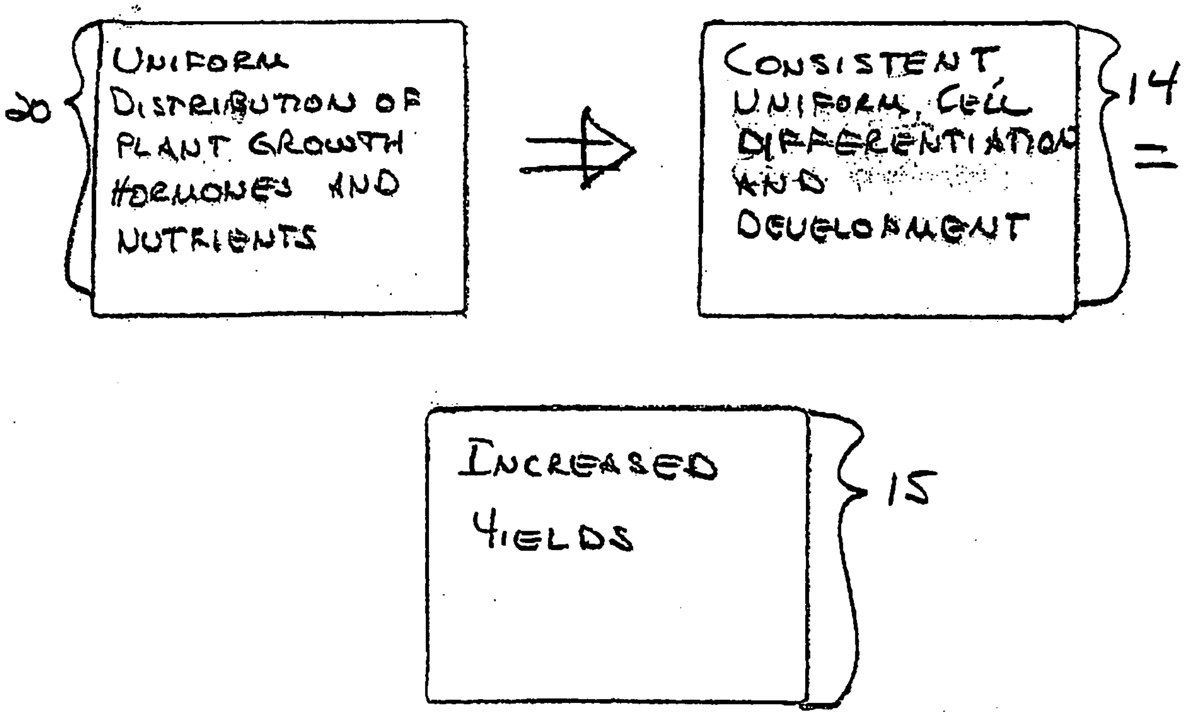

FIGURE 7B

FIGURE 7A-B

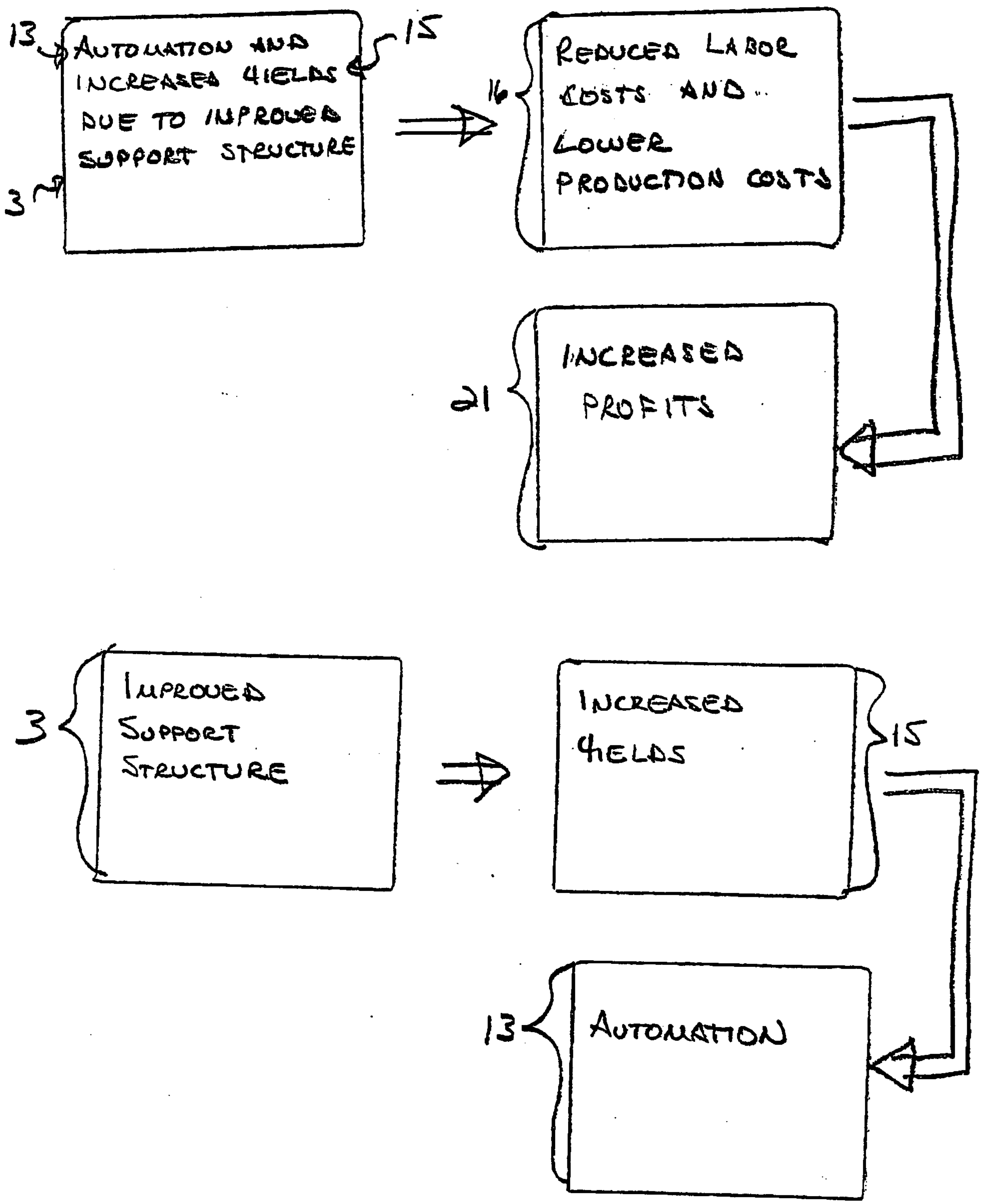

FIGURE 8A

FIGURE 8A-B